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## (54) ENZYME FOR LYSING CELLS OF DENTAL CARIES-INDUCING MICROORGANISMS

(71) We, DAINPPON PHARMACEUTICAL CO. LTD., a body corporate organised under the laws of Japan, of 25, 3-chome, Doshomachi, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to an enzyme which is capable of lysing cells of microorganisms, to a method for producing the enzyme, and to new compositions for preventing and treating dental caries, and is an improvement in and modification of the invention claimed in our prior British Patent Application No. 17125/70 (Serial No. 1,248,896).

More particularly, the present invention relates to an enzyme which is capable of lysing cells of dental caries-including microorganisms such as cariogenic streptococci and *Lactobacillus* and a method for producing the enzyme by cultivating a microorganism belonging to the genus *Streptomyces*, and to compositions for preventing and treating dental caries which contain the said enzyme.

Since it was pointed out by Miller in 1890 that dental caries might be induced by bacteria, the causes of dental caries have been studied from a microbiological viewpoint by investigators. In 1960 Fitzgerald and Keyes reported that an experimental caries was induced by streptococci in hamster (The Journal of the American Dental Association, Vol. 61, pages 9—19, 1960). Recently, it has been reported that dental plaque formation and caries development could be prevented by degrading microbially produced dextran and removing the dental plaque by using an enzyme "dextranase" (Fitzgerald *et al;* Archives Oral Biology, Vol. 13, pages 125—128, 1968, and The Journal of the American Dental Association, Vol. 76, pages 301—304, 1968). Furthermore, attempts have been made to control the growth of dental caries-inducing bacteria, by using various compounds and medicaments, and thereby to prevent or treat dental caries. There have, however, never been attempts to lyse and kill the bacteria by using enzymes.

There has been studied a method of preventing and treating dental caries by means of direct attack on the dental caries-inducing microorganisms and thereby controlling their growth. It has been found that the dental caries-inducing microorganisms, such as cariogenic streptococci and *Lactobacillus*, are a kind of microorganism which is difficult to lyse, and that they could not be lysed either by egg white lysozyme, which is known as a bacterial cell-lytic enzyme, or by enzymes produced by type cultures of several kinds of microorganisms, i.e. type cultures of *Streptomyces albus* or *Streptomyces griseus*, or a strain belonging to the genus *Flavobacterium* which are known as microorganisms which are able to produce bacterial cell-lytic enzymes.

As a result of a screening of many kinds of microorganisms existing in soil or sewage, for the purpose of finding an enzyme which is capable of lysing cells of dental caries-inducing microorganisms, it had been already found that some strains belonging to the genus *Streptomyces* produce an enzyme strongly lysing the dental caries-inducing microorganisms.

Our prior British Patent Application No. 17125/70 (Serial No. 1,248,896) provides an enzyme capable of lysing cells of dental caries-inducing microorganisms, which enzyme is produced by cultivation of a microorganism belonging to the genus *Strepto-*

[*Price 25p*]

*myces* and capable of producing the said enzyme, preferably a wild type strain of *Streptomyces diastatochromogenes* or a mutant thereof, a wild type strain of *Streptomyces farinosus* or a mutant thereof, or *Streptomyces griseus* var. H—402 or a mutant thereof.

Further study has now shown that another strain belonging to the genus *Streptomyces* possesses a superior capacity of producing an enzyme which is capable of lysing cells of dental caries-inducing microorganisms.

The present invention provides an enzyme capable of lysing cells of dental caries-inducing microorganisms, which enzyme is produced by cultivation of a wild type strain of *Streptomyces globisporus* B—1829 (ATCC No. 21553 or FERM-P No. 596).

The B—1829 strain, producing an enzyme which is capable of lysing cells of dental caries-inducing microorganisms and which has newly been isolated by the present inventors, is deposited with American Type Culture Collection, U.S.A. (hereinafter referred to as ATCC) and with Fermentation Research Institute, Agency of Industrial Science and Technology, Japan (hereinafter referred to FERM) under the numbers ATCC No. 21553 and FERM-P No. 596, respectively.

The morphological and cultural characteristics of the strain are set forth below:

(A) *Morphological properties*:

Aerial mycelium and spore: Aerial mycelium straight or wavy; no spirals, no whorls; spores spherical, 0.4 to 0.6 $\mu$ in size.

(B) *Cultural properties on various media*:

Czapek's agar: Scanty growth; aerial mycelium light brown; no soluble pigment

Glucose-asparagine agar: Abundant growth light ivory; powdery, cream-coloured aerial mycelium; soluble pigment faint yellowish brown.

Calcium malate agar: Abundant growth cream-coloured; powdery, cream-coloured aerial mycelium; soluble pigment faint brown

Nutrient agar: Abundant growth cream-coloured; scanty, white aerial mycelium; soluble pigment faint brown

Glucose nutrient agar: Abundant growth mustard-like golden; thick, powdery cream-coloured aerial mycelium; soluble pigment faint brown

Potato: Thick, mossy growth wrinkled; mossy, light grey aerial mycelium; soluble pigment brown

Glucose peptone agar: Thick growth faint golden; scanty, white aerial mycelium; soluble pigment faint brown

Starch agar: Moderate growth faint cocoa brown; thick, white aerial mycelium; soluble pigment faint brown

Gelatin stab: Very scanty growth; white aerial mycelium; no soluble pigment

Tyrosine agar: Abundant growth faint orange; powdery, cream-coloured aerial mycelium; soluble pigment faint yellow

Litmus milk: Growth with circular or thin membranous surface, light yellow; white aerial mycelium; no soluble pigment

Yeast extract-malt extract agar: Abundant growth bright golden; thick, cream-coloured aerial mycelium; soluble pigment light brown

Oatmeal agar: Moderate growth faint yellowish brown; thick, white aerial mycelium; soluble pigment light brown

Glycerine-asparagine agar: Abundant growth faint yellowish brown; thick, powdery, cream-coloured aerial mycelium; soluble pigment faint yellow.

(C) *Physiological properties*:

| | |
|---|---|
| Gelatin liquefaction | Positive |
| Starch hydrolysis | Positive |
| Tyrosinase reaction | Negative |
| Litmus milk | Peptonisation |
| Reduction of nitrate | Positive |
| Pigment producing reaction | Negative |

(D) *Utilisation of saccharide*:

| | |
|---|---|
| Arabinose | + |
| Xylose | + |
| Glucose | + |
| Mannose | + |
| Fructose | + |
| Lactose | + |
| Sucrose | ± |
| Inositol | ± |
| Rhamnose | ± |
| Raffinose | ± |
| Salicin | ± |
| Mannitol | + |

(+=moderate utilisation
±=scanty utilisation)

On the basis of these morphological, cultural and physiological characteristics, the classification of the strain has been determined according to Wakeman's "The Actinomycetes". Consequently, it seems that the B—1829 strain (ATCC No. 21553; FERM-P No. 596) should be classified as *Streptomyces globisporus*.

The enzyme according to the present invention can be produced by cultivation of a wild type strain of *Streptomyces globisporus* B—1829 (ATCC No. 21553; FERM-P No. 596). The enzyme according to the present invention can also be produced by using mutants of the said microorganism, such as

those obtained by natural mutation, or those produced by mutogenic agents, for example, X-ray irradiation, ultraviolet irradiation and nitrogen mustards.

The microorganism belong to *Streptomyces globisporus* B-1829 used according to the present invention is cultivated in a suitable culture medium containing, for example, saccharides, nitrogen sources, inorganic salts and further, if desired, organic stimulants and thereby the desired enzyme is accumulated in the medium.

Saccharides used in the culture of the enzyme according to the present invention may be, for example, glucose, maltose, malt extract, dextrin or starch. The nitrogen sources may be, for example, inorganic nitrogen sources such as ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate or potassium nitrate; and organic nitrogen sources such as urea, peptone, soybean extract, soybean meal, yeast extract or meat extract, may also be used. Inorganic salts may be, for example, sodium chloride, sodium dihydrogen phosphate, potassium dihydrogen phosphate disodium hydrogen phosphate, dipotassium hydrogen phosphate, magnesium sulphate, ferric sulphate, zinc sulphate, or calcium chloride. Furthermore, organic stimulants may be, for example, vitamins such as vitamin $B_1$ and vitamin $B_2$, peptone, meat extract, or corn steep liquor.

The pH value of the medium is preferably controlled at 6 to 9, more particularly at 7 to 8, by addition of acids, such as hydrochloric acid or acetic acid, or bases, such as sodium hydroxide, potassium hydroxide or ammonium hydroxide.

The culture can be carried out by conventional culture methods, such as stationary culture, shaking culture and submerged culture, preferably by shaking culture, at temperatures from 20 to 40°C, preferably at temperatures from 25 to 37°C. The period of the culture is from 1 to 10 days, preferably from 1 to 3 days.

The thus obtained culture broth containing the desired enzyme can be submitted to isolation, recovery and purification by means of conventional methods generally used for the production of enzymes. For example, the culture broth may be separated by centrifuging, and water or a buffer solution, such as acetate buffer, phosphate buffer, tris-maleate buffer or tris-HCl buffer, may be added to the resulting supernatant fluid to give an enzyme solution (hereinafter referred to as broth enzyme solution). Furthermore, the supernatant fluid may be conventionally purified by salting out with ammonium sulphate, precipitation by acetone, dialysis and/or phosphoric acid gel, carboxymethyl cellulose or Sephadex (Registered Trade Mark) chromatography, followed by addition of water or buffer solution to give a purified enzyme solution (hereinafter referred to as purified enzyme solution). Furthermore, these enzyme solutions can be freeze-dried to give a dried enzyme product. Both enzyme solutions and the dried enzyme product obtained as described above can be used for the preparation of compositions according to the present invention for preventing and treating dental caries.

The properties of the enzyme according to the present invention are illustrated diagrammatically in the accompanying drawings.

Figure 1 shows the relation between pH and microorganism cell-lytic activity of the enzyme when an enzyme solution produced according to the present invention is applied to cariogenic streptococci.

Figure 2 shows the relation between the temperature and the activity of an enzyme solution produced according to the present invention.

The enzyme produced according to the present invention possesses the activity of lysing cells of microorganisms over a wide range of pH values, e.g. from pH 5 to 9, as shown in Figure 1. The optimum temperature is approximately 50 to 60°C, as shown in Figure 2. Moreover, the enzyme according to the present invention is rather unstable to heat and, for example, it lost almost all its activity when it was preserved at 80°C for 20 minutes.

The unit of lytic activity of the enzyme according to the present invention and the reduction ratio of bacteria cells in turbidity by the present enzyme were calculated according to the following method.

0.4 ml of a suspension of intact cells or heated cells of microorganisms to be lysed, 2 ml of an enzyme solution diluted in appropriate concentration, and 1.6 ml of 0.025 M tris-HCl buffer (pH 7.0) were mixed to give a total of 4.0 ml. The mixture was kept at 37°C for 5 minutes to allow the cell-lytic reaction. Then the optical density of the reaction mixture was measured at 600 mμ by a photoelectric colorimeter, and the unit of lytic activity and the reduction ratio of bacteria cells in turbidity by the enzyme according to the present invention were calculated according to the following equations. One unit of activity is defined as that amount of enzyme giving an initial linear decrease in optical density of 0.001 per minute. As a control, 2 ml of water was used instead of 2 ml of the enzyme solution.

$$\text{Unit/ml} = \frac{(a-b)-(a-c)}{0.001.t.v} = \frac{c-b}{0.001.t.v}$$

a = optical density of the reaction mixture at 600 mμ at zero reaction time,

b = optical density of the reaction mixture at 600 mμ after a time t,
c = optical density of the control solution at 600 mμ after a time t,
t = reaction time (minutes)
v = volume of original enzyme solution (not diluted)
Reduction ratio =

$$\frac{\left\{\begin{array}{l}\text{Optical density of the control}\\ \text{solution after the reaction}\end{array}\right\} - \left\{\begin{array}{l}\text{Optical density of the enzyme}\\ \text{solution after the reaction}\end{array}\right\}}{\text{Optical density of the control solution after the reaction}} \times 100$$

The enzyme according to the present invention can specifically attack several kinds of dental caries-inducing microorganisms and shows superior activity in lysing cells of these. The enzyme can therefore be therefore be used for preventing and treating dental caries in humans.

Furthermore, the enzyme is also useful for prevention and treatment of the dental plaque which is induced by dental caries-inducing microorganisms and causes the dental caries. The development of the dental plaque can be controlled as a subsidiary result of prevention and treatment of dental caries by the enzyme according to the present invention.

The enzyme according to the present invention can be applied to human teeth for the purpose of preventing and treating dental caries by otherwise conventional methods, applying otherwise conventional types of unit dosages or carriers. The conventional carriers may be, for example, water, tooth powder, toothpaste, chewing gum or ointment.

In the preparation of toothpaste and tooth powder containing the enzyme according to the present invention, conventional vehicles can be used unless they have an undesirable effect on the activity of the enzyme. Suitable water-insoluble polishing agents can be employed. The polishing agent may be, for example, dicalcium phosphate, tricalcium phosphate or magnesium carbonate. These polishing agents will generally constitute a major proportion by weight of the solid ingredients. The content of the polishing agents is preferably from 30 to 60% by weight of the total composition in toothpaste, and from 85 to 95% by weight in tooth powder. The enzyme is generally employed in an amount of from 1 to 5000 units (as hereinbefore defined) per gram of the composition.

In the preparation of toothpaste, one or more plasticisers may be added to a mixture of powdery vehicles to give a paste. The plasticiser may be, for example, water, glycerol, sorbitol, propylene glycol, glycerol monostearate, white petroleum jelly or cetyl alcohol, or a mixture thereof. It may be preferable to add a gelling agent, such as sodium carboxymethyl cellulose, hydroxyethyl cellulose, polyvinyl pyrrolidone or gum tragacanth, to the composition. Furthermore, there may optionally be added other additional components such as flavouring, sweetening and colouring agents. On brushing teeth with a toothbrush or finger spread with a toothpaste or tooth powder containing the enzyme according to the present invention, the dental caries-inducing microorganisms on the teeth can be lysed by the enzyme, whilst the dental plaque is eliminated and the teeth are cleaned.

Similar effects can be achieved by using a chewing gum containing the enzyme according to the present invention.

In the preparation of chewing gum containing the enzyme according to the present invention, conventional gum bases such as chicle resin or polyvinyl acetate may be used. Other vehicles, such as plasticisers, softeners, sugar, flavouring and colouring agents may also be added. The content of the enzyme may be from 1 to 5000 units (as hereinbefore defined) per gram of the composition.

Another means of using the enzyme according to the invention is in the form of an ointment, which can be applied to the teeth to be treated, followed by rubbing with a finger or a toothbrush. In the preparation of the ointment, there may be used any conventional vehicles which can be applied to the mouth, excepting one having an inhibitory or destructive action on the enzyme. As an ointment base, there may be used materials, such as glycerol and sodium carboxymethyl cellulose, which can form jelly-like or creamy ointments. The content of the enzyme according to the present invention may be from 2 to 3000 units per gram.

The enzyme may also be applied to teeth by washing or rinsing the mouth with a mouthwash containing the enzyme. The mouthwash can contain from 0.1 to 50 units of the enzyme per millilitre. It may further contain antibiotics or other sterilisers. The mouthwash containing the enzyme may be also applied by using a spray.

After washing or rinsing the mouth, it may be preferable not to rinse the mouth with clean water since it is desirable to leave the enzyme in contact with the teeth for a long period.

The enzyme according to the present invention may be also used in a form of a chewable tablet or troche. By chewing or keeping the chewable tablet or troche containing the enzyme in the mouth, the enzyme can be sufficiently contacted with the teeth for a long period. Conventional vehicles such as mannitol and sorbitol, and other conventional additives such as lubricants, sweetening agents, colouring agents may be used in the preparation of the chewable tablet or troche. The content of the enzyme may be 1 to 5000 units in a unit dose.

The enzyme according to the present invention may also be mixed with confectionary products, such as candy or cake.

Furthermore, the enzyme may be administered in admixture with foodstuffs or beverages, the enzyme having been mixed with the foodstuffs or beverages before or after processing thereof.

The application of the present enzyme for the purpose of prevention and treatment of dental caries is not limited to the above described methods, and many other variants or modifications can be employed. It should be noted, however, that heat treatment should be avoided when preparing compositions containing the enzyme according to the present invention since the enzyme is unstable to heat and therefore, if necessary, the enzyme should be admixed after any heat treatment. The enzyme may be stabilised by adding a suitable stabiliser, such as a nonionic detergent, polysaccharide, sugar alcohol or amino acid to the compositions. Examples of the most suitable stabilisers are sucrose, mannose, sorbitol and proline.

The quantity of the enzyme used for the preparation of the compositions according to the present invention may be varied depending upon the nature of the compositions and the methods for application as described above, but may be employed in such quantites as to provide from 1 to 5000 units, more preferably 10 to 3000 units, of the enzyme per unit dose.

The enzyme of the present invention does not show any toxicity or any undesirable side effects, even in use over an extended period. If the enzyme should be swallowed, it is deactivated or decomposed in the stomach and is converted into harmless amino acids. By contrast, most antibiotics which are generally used for inhibiting various kinds of microorganisms exhibit undesirable effects on intestinal microflora.

The enzyme according to the invention has the further benefit that there is no enzyme-resistant strain of dental caries-inducing microorganisms, while the microorganisms are resistant to most antibiotics.

By means of the application of the enzyme according to the present invention, not only are cells of the dental caries-inducing microorganisms lysed, and the dental caries thereby prevented and treated, but also the teeth themselves can be made white, although the reasons for this are not clear.

The enzyme according to the present invention can lyse not only dental caries-inducing microorganism cells, but also cells of several other kinds of microorganisms, especially Gram positive bacteria, particularly those belonging to the genera *Bacillus* and *Lactobacillus*.

The preparation of the enzyme according to the present invention and of compositions containing the enzyme are set out in the following Examples. Unless otherwise noted, the percentages (%) in the compositions of the medium are by weight per volume.

*Example 1*

The isolated B-1829 (ATCC No. 21553) strain was inoculated on a slant agar medium containing 1% of glucose, 0.2% of peptone, 0.1% of yeast extract, 0.1% of meat extract and 1.5% of agar and cultivated at 30°C for 7 days. The obtained spores were inoculated into a 500 ml Sakaguchi flask including 50 ml of liquid medium (pH 7.5) containing 2% of dextrin, 0.5% of soybean powder, 0.25% of peptone, 0.5% of disodium phosphate, 0.1% of potassium dihydrogen phosphate, 0.1% of magnesium sulphate and 0.5% of sodium chloride and subjected to shaking culture at 30°C for 3 days. The obtained culture broth was separated by filtration and the resulting filtrate was diluted with distilled water to give an enzyme solution of 20 times by volume on which the unit was calculated.

Separately, 3 or 4 platinum loops of each of several kinds of cariogenic streptococcus to be lysed were inoculated into 200 ml flasks including 190 ml of liquid medium (pH 7.4) containing 2% of glucose, 1% of peptone, 1% of meat extract, 0.5% of sodium chloride, 0.2% of yeast extract, 1% of sodium acetate and $1 \times 10^{-4}$ M manganese sulphate and subjected to stationary culture at 37°C for 2 days. The cells produced were harvested by centrifuge, washed twice with water, centrifuged and freeze-dried.

To 0.4 ml of a solution which was prepared by dissolving 100 mg of the freeze-dried cells obtained above in 25 ml of distilled water were added 2 ml of the enzyme solution obtained above and 1.6 ml of 0.025 M tris-HCl buffer (pH 7.0) to give a total of 4 ml. The mixture was reacted at 37°C for 5 minutes. The optical density of the reaction mixture at 600 mμ was measured using a photoelectric colorimeter and the reduction ratio of the microorganisms to be lysed was calculated in accordance with the equation described above. The results are shown in Table I.

TABLE I

| Microorganisms to be lysed | Reduction ratio (%) 5 minutes | Units/ml |
|---|---|---|
| cariogenic streptococcus (AHT) ... ... | 30 | 248 |
| cariogenic streptococcus (BHT) ... ... | 54 | 452 |
| cariogenic streptococcus (HSR—6) ... | 57 | 475 |
| cariogenic streptococcus (HS—6) ... | 26 | 221 |
| cariogenic streptococcus (K—1—R) ... | 31 | 262 |
| cariogenic streptococcus (FA—1) ... | 45 | 379 |

*Example 2*

The isolated B-1829 (ATCC No. 21553) strain was cultivated in the same manner as described in Example 1 to provide an enzyme solution. On the other hand, intact cells of cariogenic streptococcus (BHT) were cultivated in the same manner as described in Example 1 to provide freeze-dried cells which were dissolved in distilled water to give a sample to be tested.

By using these enzyme solutions and samples to be tested, the cell-lytic reaction was carried out in the same manner as described in Example 1 and the reduction ratio of the microorganisms to be lysed was calculated. The results are shown in Table II.

TABLE II

| Volume of enzyme solution added into 4 ml of the reaction mixture (ml) | Reduction ratio (%) 5 minutes | 10 minutes |
|---|---|---|
| 0.01 | 6 | 12 |
| 0.10 | 29 | 55 |
| 0.20 | 37 | 73 |
| 0.40 | 58 | 98 |

*Example 3*

The isolated B-1829 (ATCC No. 21553) strain was cultivated in the same manner as described in Example 1. By using the obtained enzyme solution, the cell-lytic reaction was carried out on the various microorganisms in the same manner as described in Example 1 and the unit was calucalted in each instance. The results are shown in Table III.

TABLE III

| Microorganisms to be lysed | Units/ml |
|---|---|
| (1) Gram positive bacteria | |
| Cariogenic streptococcus (BHT) | 455 |
| *Streptococcus salivarius* | 272 |
| *Streptococcus lactis* | 66 |
| *Streptococcus bovis* | 456 |
| *Streptococcus faecalis* | 40 |
| *Micrococcus lysodeikticus* | 30 |
| *Sarcina lutea* | 24 |
| *Sarcina marcescens* | 4 |
| *Staphylococcus albus* | 218 |
| *Staphylococcus aureus* | 18 |
| *Bacillus subtilis* | 1040 |
| *Bacillus sphericus* | 784 |
| *Brevibacterium ammoniagenes* | 50 |
| *Lactobacillus acidophilus* | 36 |
| *Lactobacillus arabinosus* | 116 |
| *Lactobacillus brevis* | 500 |
| *Lactobacillus bulgaricus* | 236 |
| *Lactobacillus casei* | 60 |
| *Leuconostoc mesenteroides* | 434 |
| *Tetracoccus soyae* | 90 |
| (2) Gram negative bacteria | |
| *Aerobacter aerogenes* | 658 |
| *Aeromonas hydrophilia* | 86 |
| *Achromobacter liquidum* | 40 |
| *Alcaligenes faecalis* | 68 |
| *Cellulomonas flavigena* | 706 |
| *Escherichia coli* | 40 |
| *Flavobacterium esteroaromaticum* | 168 |
| *Pseudomonas fragi* | 208 |
| *Pseudomonas fluorescens* | 56 |
| *Pseudomonas aeruginosa* | 56 |
| (3) Other microorganisms | |
| *Myobacterium phlei* | 224 |
| *Candida albicans* | 32 |
| *Saccharomyces cerevisiae* | 60 |
| *Candida utilis* | 16 |

*Example 4*

Spores of the isolated B-1829 (ATCC No. 21553) strain, obtained in the same manner as described in Example 1, were inoculated into three 500 ml Sakaguchi flasks including 50 ml of liquid medium having the same composition as described in Example 1 and subjected to shaking culture at 30°C for 24 hours.

Three 3 litre flasks, each containing 1 litre of the same medium as described above, were each inoculated with 50 ml of the culture broth obtained above and then they were subjected to shaking culture at 30°C for 24 hours. The thus obtained culture broths were used as seed cultures. The culture broths were inoculated into a 100 litre fermentation tank including 70 litres of the same medium as that in the above seed culture and subjected to submerged culture at 30°C, with an air pressure of 0.5 kg/cm², an aeration rate of 70 litres/min and an agitating velocity of 150 rpm. The units of the obtained enzyme at each culture period are shown in Table IV.

TABLE IV

| Culture Period (hours) | pH | Units/ml |
|---|---|---|
| 26 | 6.92 | 80 |
| 44 | 7.08 | 348 |
| 51 | 7.20 | 534 |
| 68 | 7.38 | 840 |

*Example 5*

After 10 litres of the culture broth obtained by the same manner as described in Example 1 was separated by filtration, the filtrate was added to 400 g of the cationic ion-exchange resin Amberlite (Registered Trade Mark) CG 50. The mixture was adjusted to a pH of 5.2 to 5.5 with concentrated aqueous ammonia, agitated under cooling for one hour and filtered. The adsorbed enzyme on the resin was eluted with $\frac{1}{8}$ volume of 0.2 M disodium hydrogen phosphate aqueous solution (pH 7.5). The eluate was salted out by 60% saturation with ammonium sulphate (455 g of ammonium sulphate per litre of eluate). The resulting precipitate was dissolved in 200 ml of 0.05 M phosphate buffer (pH 7.0) and dialysed against 3 litres of the same buffer in a Cellophane (Registered Trade Mark) tube for 24 hours to provide 200 ml of solution. To the thus obtained solution was added $^1/_{10}$ volume of diethylaminoethyl cellulose, and then the mixture was filtered and freeze-dried to give 2.5 g of enzyme powder whose activity was 400,000 units/g.

*Example 6*

A toothpaste having the following prescription was prepared:

| | |
|---|---|
| Glycerol ... ... ... | 25.70% |
| Sodium carboxymethyl cellulose ... ... ... | 0.95 |
| Distilled water ... ... | 20.15 |
| Dicalcium phosphate ... | 46.00 |
| Calcium carbonate ... ... | 6.20 |
| Flavour ... ... ... | 0.25 |
| Saccharin ... ... ... | 0.25 |
| The enzyme solution obtained by Example 1, not diluted | 0.50 |
| Total | 100.00 |

*Example 7*

A tooth powder having the following prescription was prepared:

| | |
|---|---|
| Sodium lauryl sarcoside ... | 3 % |
| Sodium carboxymethyl cellulose | 1 |
| Disodium phosphate ... ... | 2 |
| Saccharin ... ... ... | 0.2 |
| Flavour ... ... ... | 1.0 |
| The enzyme solution obtained by Example 1, not diluted ... | 0.5 |
| Dicalcium phosphate ... ... | balance |

*Example 8*

A dental liquid having the following prescription was prepared:

| | |
|---|---|
| Potassium lauryl sarcoside ... | 5.0% |
| Ethyl alcohol ... ... ... | 8.0 |
| Saccharin ... ... ... | 0.3 |
| Flavour ... ... ... | 1.0 |
| The enzyme solution obtained by Example 1, not diluted ... | 0.3 |
| Distilled water ... ... ... | balance |

*Example 9*

A chewable tablet having the following prescription was prepared:

| | |
|---|---|
| The enzyme solution obtained by Example 1, not diluted ... | 0.3% |
| Corn starch ... ... ... | 10 |
| Talc ... ... ... ... | 2 |
| Saccharin ... ... ... | 0.5 |
| Flavour ... ... ... ... | 0.2 |
| Sorbitol ... ... ... | balance |

*Example 10*

A freeze-dried product of 2 ml of the enzyme solution obtained by Example 1, not diluted with water, 10 g of sorbitol powder, 360 g of disodium hydrogen phosphate ($Na_2HPO_4.12\,H_2O$), 140 g of potassium dihydrogen phosphate ($KH_2PO_4$), 1 g of saccharin and 0.5 g of flavour were mixed to give a powdery mixture. The mixture may be diluted in 200 times with distilled water before the use and then applied orally.

WHAT WE CLAIM IS:—

1. An enzyme capable of lysing cells of dental caries-inducing microorganisms, which enzyme is produced by cultivation of a wild-type strain of *Streptomyces globisporus* B-1829 (ATCC No. 21553 or FERM-P No. 596).

2. An enzyme as claimed in claim 1 substantially as hereinbefore described.

3. A method for producing an enzyme according to claim 1 which comprises cultivating a wild-type strain of *Streptomyces globisporus* B-1829 (ATCC No. 21553 or FERM-P No. 596) in a culture broth and recovering the produced enzyme from the culture broth.

4. A method as claimed in claim 3 wherein cultivation is carried out by shaking culture at 20 to 40°C for a period of from 1 to 10 days.

5. A method as claimed in claim 4 wherein the temperature is from 25 to 37°C.

6. A method as claimed in claim 4 or 5 wherein cultivation is carried out for a period of from 1 to 3 days.

7. A method as claimed in any of claims 4 to 6 wherein cultivation is carried out at a pH value of from 6 to 9.

8. A method as claimed in claim 7 wherein the pH value is from 7 to 8.

9. A method as claimed in claim 4 substantially as hereinbefore described.

10. A method as claimed in claim 4 substantially as described with reference to any of Examples 1 to 5.

11. Enzymes when produced by a process as claimed in any of claims 4 to 10.

12. A composition for preventing and treating dental caries which comprises as an essential active ingredient the enzyme as claimed in any of claims 1, 2 or 11 in admixture with a carrier.

13. A composition as claimed in claim 12 wherein 1 to 5000 units (as hereinbefore defined) of the enzyme is contained in a unit dose.

14. A composition as claimed in claim 13 wherein 10 to 3000 units (as hereinbefore defined) of the enzyme are contained in a unit dose.

15. A composition as claimed in any of claims 12 to 14 wherein the carrier is water or a toothpaste, tooth powder, chewing gum, ointment or confectionary product.

16. A composition as claimed in any of claims 12 to 14 in the form of chewable tablet or troche containing 1 to 5000 units (as hereinbefore defined) of the enzyme.

17. A composition as claimed in any of claims 12 to 16 containing a stabiliser.

18. A composition as claimed in claim 17 wherein the stabiliser is sucrose, mannose, sorbitol or proline.

19. A composition as claimed in claim 12 substantially as hereinbefore described.

20. A composition as claimed in claim 12 substantially as described with reference to any of Examples 6 to 10.

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Agents for the Applicants.

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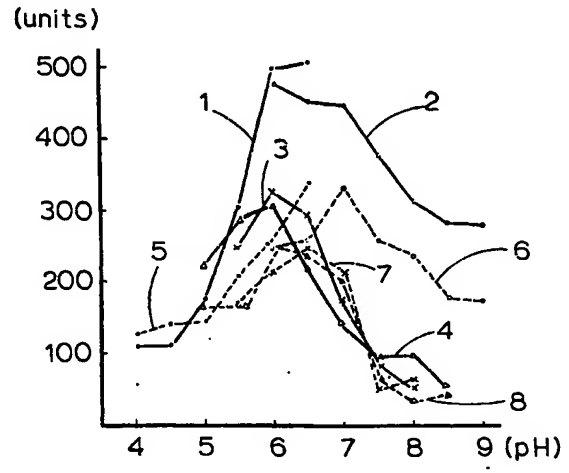
1294767 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

Fig. 1.




—— Cell-lytic curve in freeze-dried streptococcus (BHT)

---- Cell-lytic curve in heat-treated streptococcus (BHT)

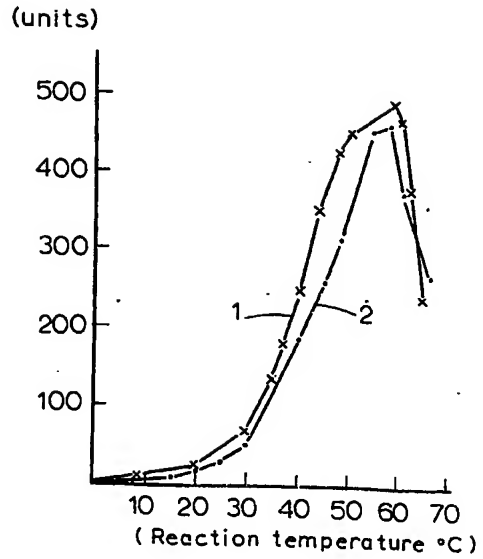
1 and 5: Acetate buffer

2 and 6: Tris-HCl buffer

3 and 7: Phosphate buffer

4 and 8: Tris-maleate buffer

Fig. 2.




1: Cell-lytic curve in freeze-dried streptococcus (BHT)
2: Cell-lytic curve in heat-treated streptococcus (BHT)